Remarks

The application now comprises claims 1-23. Independent claims 1, 8, 9 and 20, and dependent claims 2 and 12 have been amended to clarify that one or more polymerization initiation sites are formed at controlled locations on the biomolecule and the polymerization initiates from those user established sites to create a conjugate with a single biomolecule or protein. The examiner's attention is directed to paragraphs 0012, 0013, 0031, 0033, and examples 1-4 of the specification and Figures 1, 4, 7, 8 in support thereof. Claims 6 and 7 have been amended to clarify the method of obtaining the purified conjugate. The examiner's attention is directed to paragraphs 0012, 0054, and Examples 2 and 4 of the specification in support thereof. Claim 12 has also been modified to clarify that the polymerization and not the initiation reaction is in the presence of the functionalized resin. See paragraph 0041, 0042 and Figure 4.

Objection to the claims

Claims 15-19 were objected to as being dependent on a rejected base claim but were otherwise found to be allowable. Accordingly, claim 15, previously dependent on claim 9, has been amended to include original claim 9 and is now in form for allowance. Accordingly, it is requested that Claims 15 and claims 16-19 dependent thereon be indicated as allowed. Claim 12 was objected to as it contains a typographical error. The claim has also been amended to eliminate the error.

Support in the PCT Application

Claims 11, 12 and 21-23 were indicated as not having sufficient support in the PCT application PCT/US04/33125 and were therefore given an effective US filing date of April 14, 2006 which is the date of the filing of the present US application. Applicant respectfully traverses such conclusion.

Applicants contend that claims 11, 12, 21 and 22 are in fact supported by the PCT application and therefore have benefit of its filing date.

In regard to claim 11 it is known that cysteine, a natural amino acid found in proteins, provides thiol sites in that when two cysteines joined together as a disulfide are reduced a free cysteine is generated. In other words, a free thiol is created. The claim is to the modification of

the protein to contain one or more free thiol sites available for conjugation. The examiner's attention is directed to the following portions of the PCT application:

The Abstract and paragraph [0012] set forth that "the protein can be modified to contain site(s) for initiation by recombinant methods, chemical ligation, solid-phase synthesis, or other techniques to generate sites for initiation."

It is stated in paragraph [0014] (page 4 that "Cysteine residues naturally or artificially present on the protein are an example of anchoring points to modify the protein with initiator", in paragraph [0020] the protein can be modified by other techniques to react with the monomer and in paragraph [0031] that "proteins may be modified to have an initiation site or prepared with an initiation site by incorporation of amino acids suitable for polymer initiation.

In addition, Claim 1 claims reacting a monomer with a protein modified to include polymerization initiation sites, Claim 2 claims the activated site is an amino acid modified by an initiator or an initiator is added to the protein to provide an initiator site and Claim 6 claims a method of forming a protein-polymer conjugate comprising modifying the protein to be reactive with the monomer.

Taken alone or in combination these disclosures in the PCT application support claim 11.

In regard to claim 12, a known method to generate free cysteines is by reduction with a reagent such as tris-2-carboxyethyl phosphine hydrochloride and maleimide is a known way to cap a free cysteine. In addition, the use of a pyridyl disulfide initiator is described in the PCT application. The examiner's attention is directed to the following portions of the PCT application:

The abstract, Figure 1 and Example 1, Pages 7 & 8 describe formation of a pyridyl disulfide initiator (BSA initiator BSA –I) and pages 5 and 7 refer to the formation of a disulfide bond and conjugation via a disulfide bond. Claim 2 claims the activated site is an amino acid modified by an initiator or an initiator is added to the protein to provide an initiator site and claim 8 claims a pyridyl disulfide initiator.

Claim 21, dependent on claim 2, sets forth that the biomacromolecule is an enzyme and Claim 23, dependent on claim 2 sets forth that the biomacromolecule is an antibody. Such claimed invention is supported by the PCT specification at paragraphs [0026] and [0038]. In particular paragraph [0038] of the PCT application states that

> the methods described in the application "can also be applied to the formation of polymer conjugates with a wide range of biomolecules such as enzymes or antibodies.

Accordingly, it is submitted that claims 21 and 23 have the benefit of the PCT filing date, namely October 7, 2004. Further, it is submitted that claims 21 and 23 have the benefit of the Provisional Application filing date (Oct. 15, 2003) as substantially the same language appears in Example 1 on page 3 thereof. In addition, while the use of lysozyme was not specifically disclosed in the PCT application or Provisional Application, lysozyme is an enzyme and is therefore within the scope of the disclosure therein and claim 22 likewise has the benefit of the PCT application filing date.

35USC §102(a)

Claims 11, 12, 21 and 22 were rejected under 35 USC §102(a) as anticipated by Heredia et la (J. Am. Chem. Soc, 127, No 47, pp 16995-16960 (Nov 30, 2005) (it is believed the reference should have been to pages 16955-16960) in that,

a) as to claims 11 and 12 Heredia et al. teaches forming a protein polymer conjugate comprising modifying the protein to have functionality suitable for initiation of radical polymerization; reacting the protein with a monomer; reducing the protein with tris (2-carboxyethyl) phosphine hydrochloride to produce additional thiols on the protein, modifying the protein with pyridyl disulfide in the presence of 2-bromoisobutyrate functionalized resin, capping the unmodified thiols with maleimide to form the macroinitiator and reacting the macroinitiator with a monomer to form the conjugate, and

b) as to claims 21 and 22 Heredia et al. teaches a method of forming a polymer-biomolecule conjugate comprising reacting a monomer with sites on a lysozyme that have been modified to include polymerization initiation sites.

As to the rejection of claims 11 and 12 based on Heredia, claim 12 has been amended to clarify that the polymerization, and not the initiator reaction may be in the presence of the 2-bromoisobutyrate functionalized resin.

Irrespective of said application having the benefit of the PCT application filed July 10, 2004 and the Provisional Application filed October 15, 2003, it is respectfully submitted that Heredia et al. is not prior art, under §102(a), to the application filed in the US on April 14, 2006 in that the publication is not showing that the invention "was known or used by others ...or described in a printed publication before the invention thereof by the applicant for patent" because the publication is a publication by applicants, not others. The article lists the inventors Bontempo and Maynard as authors; the other authors (Heredia, Ly, Byers and Halstenberg) assisted the inventors by preparing the conjugates according to the inventors instructions and the testing of the invention but they did not conceive or reduce the invention to practice and are therefore not inventors. Bontempo and Maynard have previously submitted a sworn Declaration that they are the joint inventors of the claimed invention and there are no other inventors. The cited document is a publication by the inventors less than one year prior to the filing date of the subject US application.

In regard to claims 11, 12 and 21-23, the examiners attention is also directed to the above response regarding the support for the claimed invention in the PCT Application, filed July 10, 2004 and the Provisional application filed October 15, 2003, both of which were significantly prior to the publication date of the Heredia et al. article.

<u>35 USC§102(b)</u>

Claims 1-3, 5, 8, 9, 13 and 21 were rejected under 35 USC §102(b) as anticipated by Gololobov et al. (US Patent 6,433,078) in that, as to claims 1, 8 and 21 Gololobov et al. teaches a method for forming a polymer-enzyme/biomolecule conjugate comprising reacting a monomer with sites on the enzyme modified to include polymerization initiation sites, as to claims 2 and 3 Gololobov et al. teaches an enzyme/protein including amino acids, as to claim 5 Gololobov et al. teaches filtering the conjugate and removal of unreacted starting materials, as to claim 9 Gololobov et al. teaches a method for forming a polymer-enzyme/protein conjugate comprising

reacting a monomer with sites on the enzyme modified to include vinyl groups/functionality for radical initiation, and as to claim 13 Gololobov et al. teaches the monomer being isopropylacrylamide.

It is acknowledged that Gololobov et al. teaches formation of biomolecule conjugates. However, he does so in an entirely different way and as a result he does not have control over the specificity of the conjugation site, generates a variety of different conjugates from a single reaction, creates numerous undesirable side products and gels, produces conjugates including more than one biomolecule and, as a result, has a poor efficiency of reproducibly generating a desired, specific end product. Gololobov et al. is directed to the modification of enzymes with vinyl groups to produce enzyme monomers which then act as co-monomers in a conventional radical polymerization. An initiator is added to the solution to start the polymerization. The vinyl modified enzymes are comonomers, not initiators.

Gololobov does not show or suggest formation of initiator sites on the biomolecules. Once initiated by addition of initiators to the reaction mixture the growing chain reacts with both small molecule monomer (such as NIPAAm) and one or more monomer-modified enzyme monomers. In contrast, the present invention utilizes the protein as the initiator, thus growing the polymer from one or more specific user established sites on the protein. In particular, claims 1-3 claim biomolecules which include polymerization initiation sites. Such biomolecules do not exist in Gololobov et al. Applicants' polymerization proceeds from the protein and without the protein, the polymerization would not proceed. Thus, every polymer created contains only one protein; the polymer conjugate cannot form unless it is attached to the protein as it is grown from the initiator site on the protein.

Further, Gololobov shows less than 100% incorporation of enzyme in many cases because polymer without enzyme can form (Example 6). In addition, the reference shows that more than one enzyme could be incorporated per polymer chain (Experiment 6). With applicants' claimed invention, only one protein is incorporated per polymer chain because polymerization starts on the protein and proceeds from the protein. Finally, the Gololobov process also results in gel formation (Example 6) because "nascent polymer can attach

simultaneously to more than one molecule of the modified enzyme, thereby causing the formation of a stable insoluble gel due to cross-linking." In applicants' claimed process this does not occur because the polymer is grown from a specific site or sites on the protein and multiple proteins can not exist in a single biomolecule/polymer conjugate.

As a further distinction, because Gololobov modifies amines using a modifying agent containing a vinyl group this reaction is not site selective or specific. As indicated at Col. 15, line 43: "a maximum of about 10 modified amino groups per molecule" can result. In contrast applicants' claimed process allows selective targeting of sites such as free cysteines and biotin binding pockets. As set forth at page 4 of applicants' specification, control over the number and placement of polymer chains on the amino acid sequence can be achieved.

The Gololobov et al. initiating agent is a small molecule, not a protein, and the process proceeds by contacting an initiating agent or agents with the monomer. On the other hand in applicants' claimed invention the protein is the initiator or initiation is started on the protein and then the polymerization proceeds. In Gololobov et al. (col 20, line 63-67) it is stated that "polymer chain growth and protein binding occur simultaneously." While it is possible that their added initiator will react with the vinyl enzyme first and then polymerize monomer, this is very unlikely because the monomer exists in much higher concentrations in the solution and the initiator would have a much greater propensity to bind to the monomer in high concentrations rather than vinyl enzyme which exists in low concentrations. It would be even more unlikely for every vinyl enzyme in a particular reaction to bind first to the initiator. As a result, the yield of conjugated biomolecules with the biomolecule at one end of the polymer chain or chains in Gololobov is likely to be very low. Because the polymerization in applicants' process starts from initiator sites on the protein the resultant yield of conjugated biomolecules is considerably higher (usually greater than 65% and often 100%).

The patent office also rejects these claim based on (Gololobov 4:36-43) including polymerization initiation sites or (for Claim 9) vinyl groups/functionality for radical initiation. The vinyl group in itself would not initiate the polymerization. It acts as monomer. ATRP has been conducted with initiators that contain the initiation site and a vinyl moiety (examples are

provided in the Matyjaszewski pgs 2933, 2952-2954) and no polymerization of the vinyl segment is observed. Thus, the vinyl groups described in this patent do not represent initiators. The vinyl group described in the Gololobov patent may potentially be transformed into something that could form an initiator but that would require many steps and harsh conditions detrimental to the protein such that bioactivity would likely be reduced or eliminated.

Accordingly, it is respectfully submitted that independent claims 1 and 9, as amended, are patentable as they are clearly distinguishable from the cited reference, and Gololobov et al. does not show or suggest the claimed invention. Claims 2, 3, 5 and 21 dependent on claim 1 and claim 13 dependent on claim 9 are likewise patentable.

Claims 1-3 and 5 were rejected under 35 USC §102(b) as anticipated by Kroner et al. (US 5,260,396) in that Kroner et al. teaches a method for forming a polymer-protein conjugate comprising reacting a monomer with sites on the protein modified to include polymerization initiation sites and the removal of unreacted starting materials.

Kroner et al. is directed to the incorporation of multiple proteins into materials by polymerizing a mixture of a protein, a monomer and an initiator and results in the protein being incorporated into a growing polymer by chain transfer to natural amino acids in the protein. The protein acts as a cross-linker in the reaction between the growing polymer chains as a result of random reactions of the growing polymer chains with the amino acid residue. Specifically, Kroner et al. adds the protein to the reaction solution without reactivity-enhancing modification or the creation of initiator sites on the protein. While Kroner states that the proteins used in the graft polymerization may be chemically modified, they are modified solely to change the properties of the resultant material or to help solubilize the protein. None of the modifications described at page 7-9 of Kroner include modifications that would introduce an initiator site into the protein or for that matter create a polymerizable unit (a monomer).

This is distinctly different from applicants claimed invention in which a protein is modified to produce an initiation site from which the polymer chain is grown by radical polymerization and in which the protein is at the end of the polymeric conjugate. In applicants'

claimed process the protein is modified in a controlled manner and at selected locations to have a specific initiation site or sites.

Accordingly, Kroner et al. does not show or suggest the invention of claim 1 or claims 2, 3 and 5 dependent thereon.

35USC§103 (a)

Claims 4 was rejected under 35 USC §103(a) as obvious based on Golobov et al. (US Patent 6,433,078) as applied above in regard to claim 3 in light of Matyjaszewski et al. (Chem. Rev. 2001, 101, 2921-1990) in that while Gololobov does not teach a modified polymerization site as being an initiator, Matyjaszewski et al. teaches an atom transfer radical polymerization with the initiator being attached to a macromolecule and Gololobov et al. and Matyjaszewski et al. are combinable as they are concerned with a similar technical difficulty, namely grafting acrylate monomers onto a macromolecule . The examiner contends that it would have been obvious to a person having ordinary skill in the art at the time of the invention to have used the initiator of Matyjaszewski et al. as the polymerization site of Gololobov et al. and the motivation to do so would have been, as Matyjaszewski et al. suggest, atom transfer radical polymerization which provides a uniform growth on all chains.

First, Claim 4 is dependent on allowable claim 1 and as such is also allowable. The arguments set forth above regarding Gololobov are reasserted. Further, Matyjaszewski et al. at, pages 2923-2924 teaches atom transfer radical polymerization of monomers including the use of an initiator, and suggests attaching the initiating moiety to macromolecular species with reference to US Patent 5,789,487. A reading of the Matyjaszewski '487 patent clearly shows that the examiner's contention is without basis. The definition of macromolecule provided on page 7, 14-19 of that patent is "a molecule containing a large number of monomeric units and having an average molecular weight (Mn) of at least 500"; the term "macroiniatior" refers to a macromolecule having a least one polymerizable site." Still further on the same page a "monomer for polymerization" is described but none of the examples provided can be an amino acid. Based on the '487 patent definitions one can not include a protein as a Matyjaszewski macromolecule. Accordingly, it would not be obvious to a apply the Matyjaszewski reference

alone or in combination with Gololobov to form a biomolecule conjugate and if the combination were made the resultant polymeric material would be completely different from the applicants' claimed conjugate.

Matyjaszewski et al, like Gololobov is, referring to polymerization of vinyl containing polymers and the monomer had to first be reacted with a macromolecular species that contained the initiator to attach the initiator to the monomer. He did not contemplate that the initiator site was created from the constituents of the biomolecule (i.e. cysteine which is part of the biomolecule.

The Gololobov patent is specifically directed to modifying amine groups within a protein with a vinyl group. This protein with a vinyl group is then mixed with monomer. Initiator is *added* to effect the polymerization; the initiator is not a part of the protein. As a result, the material generated is heterogeneous. This is quite distinct from applicants' invention which results in a well-defined protein-polymer conjugate as a result of the polymerization proceeding from one or more defined places on the protein. Thus, there is only one protein per polymer. The resulting material is patentably distinct from what might be possible with a procedure combining Gololobov and Matyjaszewski, and thus has much greater utility for certain applications including protein therapeutics.

Further, the teachings of Gololobov et al. and Matyjaszewski et al. are not combinable. Despite the fact that ATRP small molecule initiators have been known since their discovery in 1995 it is not obvious to modify them with initiators. Many of them have alkyl halide groups that could be displaced during the coupling of the initiator to the protein. Furthermore, it is not obvious based upon potential difficulties with non-specific chain transfer (chain transfer means non-discriminate reaction of the growing polymer chain with various functional groups on a protein which is the basis for the Kroner patent) that initiating a polymerization from a specific site on a protein would be possible. In the Gololobov patent, the growing polymer chain reacts with numerous vinyl sites on the protein and the particular vinyl groups and the number of vinyl groups it reacts with is not controllable. In contrast thereto in applicants' claimed invention all the groups initiate and grow only from sites that are controlled by the placement of initiator.

Claim 10 was rejected under 35 USC §103(a) as obvious based on Golobov et al. (US Patent 6,433,078) as applied above in regard to claim 9 in light of Matyjaszewski et al. (Chem. Rev. 2001, 101, 2921-2990) in that while Gololobov does not teach a modified polymerization site as being an initiator, Matyjaszewski et al. teaches an atom transfer radical polymerization initiator as being attached to a macromolecule where the initiator functionality is preferably 2-bromoisoburtyrate.

First, Claim 10 is dependent on allowable claim 9 and as such is also allowable. The arguments set forth above regarding combining Golobov and Matyjaszewski et al. to render obvious claim 4 are reasserted. The combination does not show or suggest modifying a protein itself to have initiator sites suitable for initiation of polymerization. They require that a compound be provided that includes an initiator site and the biomolecule be reacted with that compound to attach a pendent initiator thereto. In particular, the claim was rejected because Gololobov and Matyjaszewski both concern "grafting acrylate monomers onto a macromolecule." Claim 10 is directed to a particular method for placing monomers, not grafting, which requires growing a polymer chain or chains from a one or more specific sites, to form an A-B block or ABn star conjugate , i.e., a biomolecule/polymer conjugate with a single protein. The cited references do not render it obvious that a polymer could be grown from a particular site of a protein and not react non-discriminate, as with chain transfer, with other parts of the protein. Combining Golobov and Matyjaszewski et al. can not and does not result in applicants' claimed invention.

Claim 14 was rejected under 35 USC §103(a) as obvious based on Golobov et al. (US Patent 6,433,078) as applied above in regard to claim 9 in light of Matyjaszewski et al. (Chem. Rev. 2001, 101, 2921-2990) and Jansen et al. (US 4,980,457) in that while Gololobov does not teach a modified polymerization site as being an initiator, Matyjaszewski et al. teaches an atom transfer radical polymerization initiator as being attached to a macromolecule where the initiator functionality is preferably 2-bromoisoburtyrate. While Gololobov et al. does not teach attaching the functional group through the propyl mercapto pyridine group of the instant claim the examiner contends that Jansen et al. teaches attaching functional groups to a polymer through a

disulfide group activated by a pyridine group to allow the functionalizing agent to react with the thiols of the protein.

First, Claim 14 is dependent on allowable claim 9 and as such is also allowable. The arguments set forth above regarding Golobov and Matyjaszewski et al. are reasserted. The combination does not show or suggest modifying a protein itself to have initiator sites suitable for initiation of polymerization. They require that a compound be provided that includes an initiator site and the biomolecule be reacted with that compound to attach a pendent initiator thereto. Janssen is directed to a different reaction scheme and the suggested combination of the three references would be incompatible. While they might be alternative approaches they are not additive and cannot be combined. In any event the reaction schemes suggested therein are different from the invention set forth in claim 14.

Claim 20 was rejected under 35 USC §103(a) as obvious based on Golobov et al. (US Patent 6,433,078) as applied above in regard to claim 9 in light of Matyjaszewski et al. (Chem. Rev. 2001, 101, 2921-1990) in that while Gololobov does not teach a modified polymerization site as being an initiator, Matyjaszewski et al. teaches reacting an atom transfer radical polymerization with an initiator being attached to a macromolecule.

The arguments set forth above regarding Golobov and Matyjaszewski et al. are reasserted. The combination does not show or suggest modifying a protein itself to have initiator sites suitable for initiation of polymerization. They require that a compound be provided that includes an initiator site and the biomolecule be reacted with that compound to attach a pendent initiator thereto.

Claims 22 and 23 was rejected under 35 USC §103(a) as obvious based on Gololobov et al. (US Patent 6,433,078) as applied above in regard to claims 2 and 21 in light of Hoffman et al. (US 5,988,588) in that while Gololobov et al. does not teach the enzyme as being lysozyme, Hoffman et al. teaches using lysozyme in a polymer-biomolecule conjugate.
Claims 22 and 23 are dependent on claim 2. It is respectfully submitted, as set forth above, that claim 2 is patentably distinct from Gololobov. Accordingly claims 22 and 23 dependent on claim 2 are likewise patentable and Hoffman et al. does not provide the teachings missing in

Gololobov et al. to render claim 2 or the claims dependent thereon obvious. It is irrelevant that Hoffman suggests lysozyme is pH sensitive.

Claims 4, 6 and 7 were rejected under 35 USC §103(a) as obvious based on Kroner et al. (US Patent 5,260,396) as applied above in regard to claim 3 in light of Matyjaszewski et al. (Chem. Rev. 2001, 101, 2921-1990) in that while Kroner does not teach a modified polymerization site as being an initiator, Matyjaszewski et al. teaches an atom transfer radical polymerization with an initiator being attached to a macromolecule and, in regard to claims 6 and 7 Kroner teaches adding a water-insoluble non-interactive initiator to remove the remaining monomers from the mixture.

Claims 4, 6 and 7 are dependent on claim1. For the reasons set forth above, claim 1 is not shown by Kroner and the arguments set forth above regarding Kroner are reasserted. A combination with Matyjaszewski et al. does not render claim 1 obvious and therefore the claims dependent thereon are not rendered obvious.

In particular, in regard to claim 6, Kroner utilizes the water insoluble initiator for completing the polymerization by removing remaining monomers from the latex. The water insoluble initiator is added last. This is quite distinct from the use of the non-interacting initiator in the claimed invention wherein the non-interacting initiator is added with the protein initiator or even before. The non-interacting initiator does not complete the polymerization, but allows an increase of the over-all equivalents of initiator such that smaller amounts of protein initiator can be utilized. Thus, the interacting and non-interacting initiators are added simultaneously. In regard to claim 7, a purified protein-polymer conjugate is obtained by simultaneously creating the protein conjugate and polymerizing non-conjugated monomer made by the method of claim 6 and removing that non-conjugated material. This process is different from that suggested by a combination of Kroner and Matyjaszewski et al.

It respectfully submitted that independent claims 1, 8, 9 and 20 have not been shown to be anticipated by Gololobov et al. or Kroner et al. and are therefore patentable. Accordingly, no combination of either of these references with additional references can render obvious the claims dependent thereon. Claims 15-19 were previously indicated as allowable if written in

independent form; such amendment has been made. As the claims currently pending are not shown by any of the cited references or obvious based on a combination of those references, they are all patentable and a Notice of Allowance is respectfully requested.

Respectfully submitted,

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